A method for site-specific labeling of multiple protein thiols

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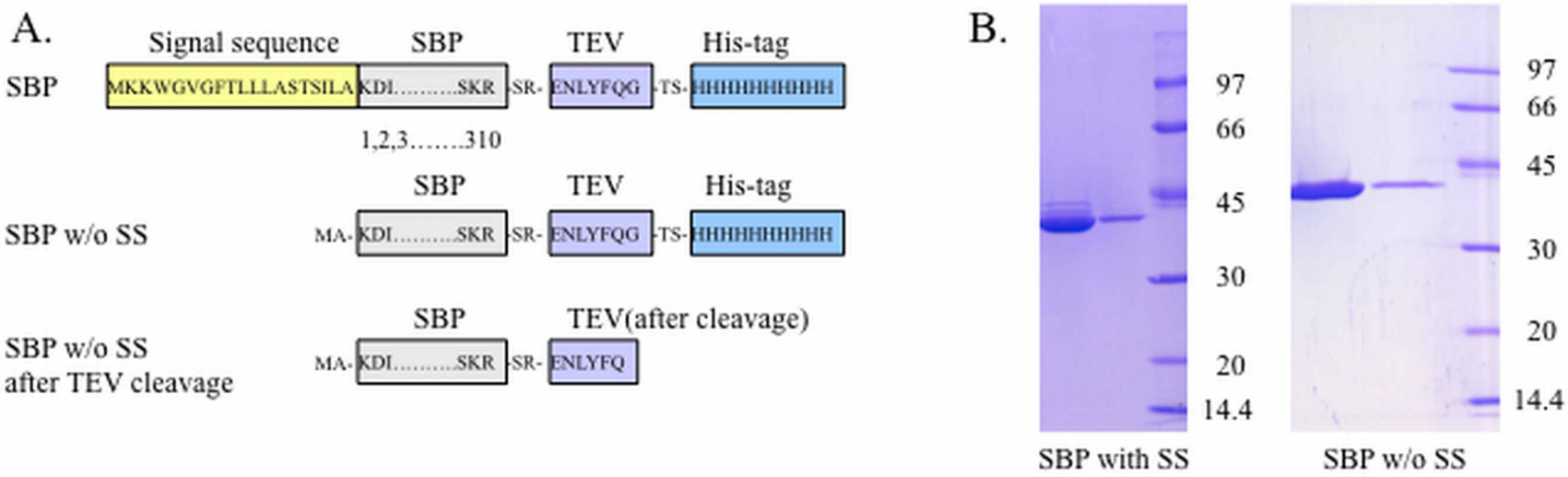
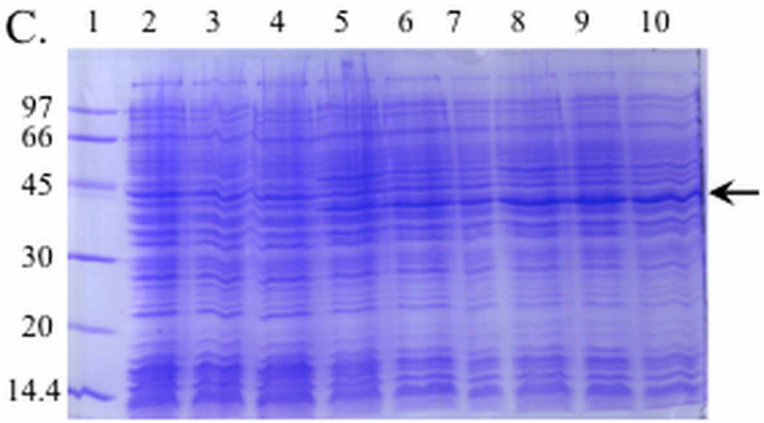
Supplementary materials: Kuiper *et al* (2008) A method for site-specific labelling of multiple protein thiols



Panel A: *The amino acid sequence of sulphate-binding protein (SBP) with and without signal sequence (SS) and after cleavage with tobacco etch virus (TEV) protease. The amino acid numbering for SBP is given below the sequence at the top.*

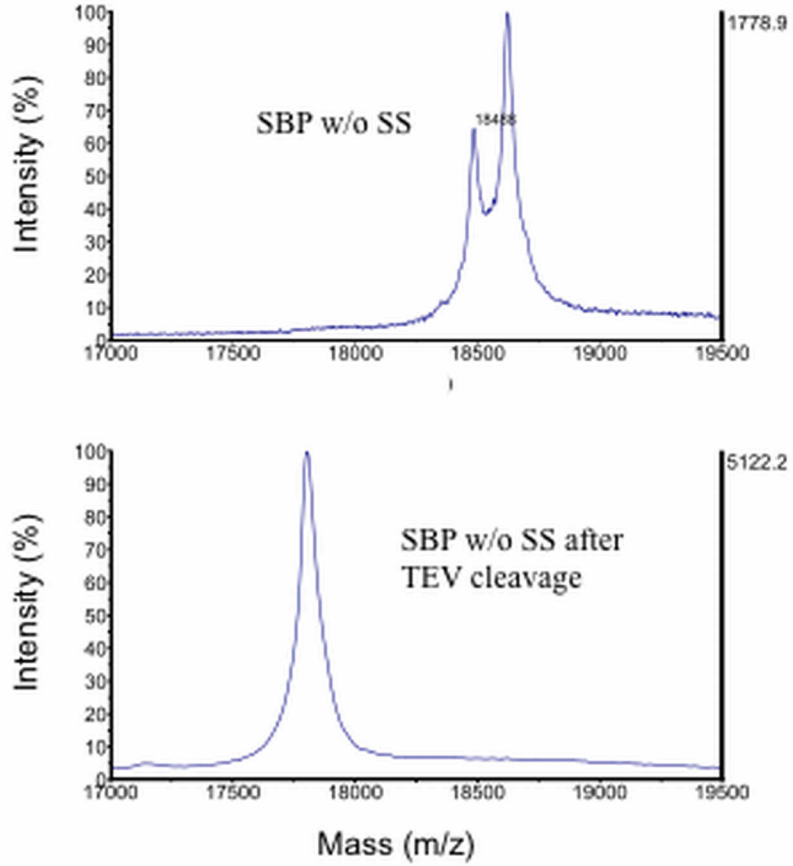
Panel B: *Coomassie Brilliant Blue (CBB)-stained 12.5% SDS-PAGE gels of SBP expressed with and without signal sequence. The numbers at the right refer to the masses of the proteins in the marker lane, expressed in kDa. Clearly visible are the extra bands at higher masses for the protein expressed with SS. Expression of the protein without SS led to a single band in SDS-PAGE.*



Panel C: *Expression of SBP. E. coli MC 1061 containing pJMK1 was cultivated until OD660=0.5. Protein expression was induced by adding different amounts of L-(+)-arabinose (Ara). Samples were taken at time zero (start of induction), t=45 min and t=90 min. The cells were harvested by centrifugation and were heated to 100oC for 4 min and total cell lysates were analyzed by SDS-PAGE. Lane 1: Marker proteins; The numbers at the left refer to the masses in kDa.; Lane 2: t=0 & 0.001% Ara; Lane 3: t=0 & 0.01% Ara; Lane 4: t=0 & 0.1% Ara; Lane 5: t=45 & 0.001% Ara; Lane 6: t=45 & 0.01% Ara; Lane 7: t=45 & 0.1% Ara; Lane 8: t=90 & 0.001% Ara; Lane 9: t=90 & 0.01% Ara; Lane 10: t=90 & 0.1% Ara. Clearly, upon induction with Ara, a protein with an apparent mass of ~38 kDa becomes visible after 45 and 90 min (see arrow).*

Supplementary materials: Kuiper *et al* (2008) A method for site-specific labelling of multiple protein thiols

D.



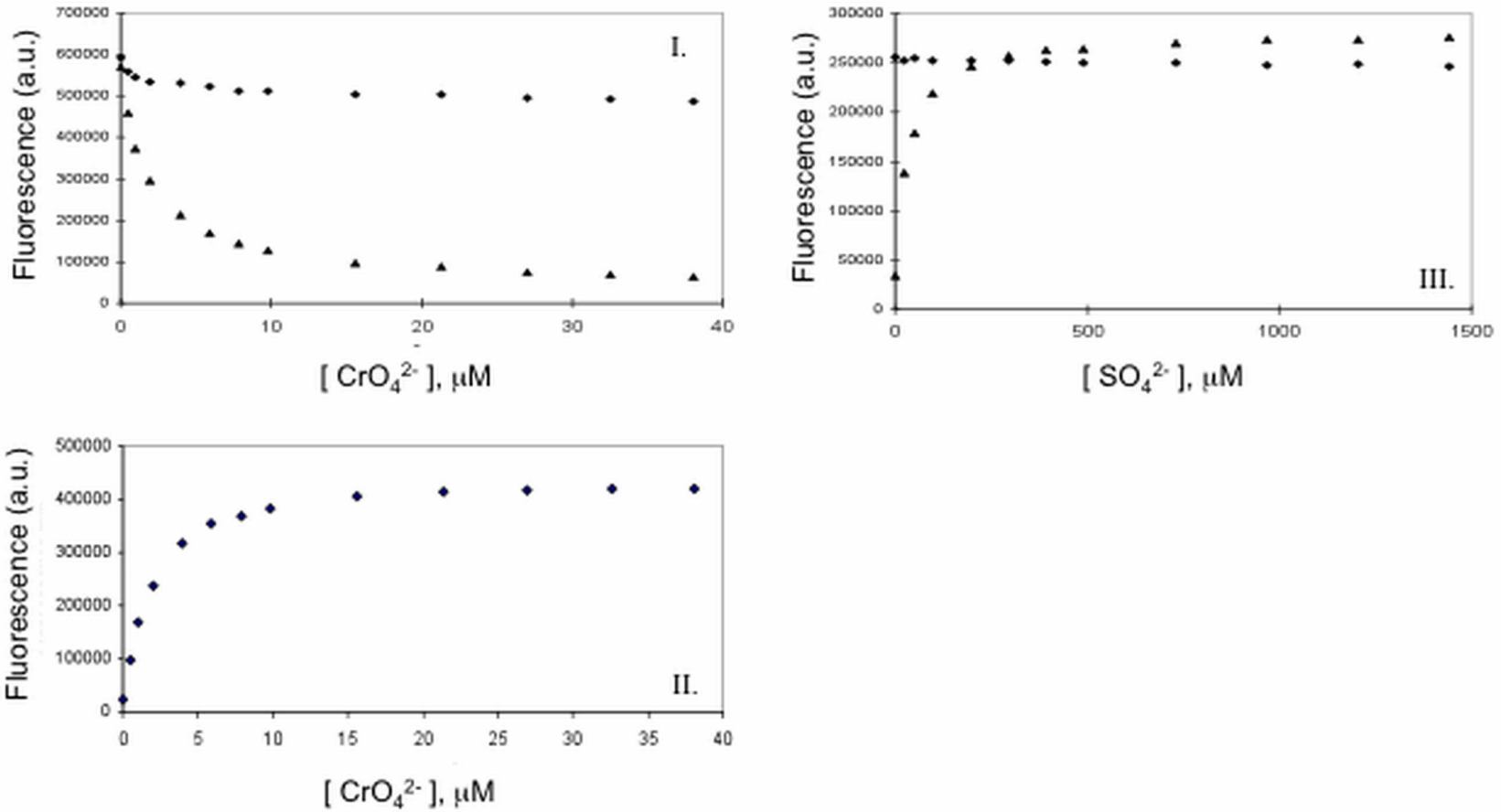
E.

	Expected M^+	Expected M^{2+}	Observed M^{2+}
SBP w/o SS	37,379	18,689	18,611 18,479
SBP w/o SS after TEV	35,762	17,880	17,805

***Panel D**: Part of the MALDI-Tof spectra of the wildtype SBP protein before and after treatment with the TEV protease. Left to the main peak of the M^{2+} ion, an extra peak at 18,488 amu is observed. After treatment with TEV, the extra peak is no longer observed (lower panel), indicating some heterogeneity at the C terminus of the protein. The data from the MS analysis are summarized in panel E.*

***Panel E**: Summary of MS data for SBP w & w/o signal sequence; M^+ and M^{2+} refer to the singly and doubly charged protein species.*

F.



***Panels F**: Ligand binding data for the determination of the K_D of SBP for CrO_4^{2-} and SO_4^{2-}. **Subpanel I**. To 1 mL of 2 mM Tris-HCl, pH8.0, 1 mM EDTA was added 10 μL of 53 μM SBP. Subsequently, CrO_4^{2-} from 1 mM stock solutions was added under continuous stirring (▲). The control measurements were performed with buffer (♦). **Subpanel II**. The difference in fluorescence between SBP and control sample was plotted against the total concentration of CrO_4^{2-}. The data was fitted with the general binding equation (Lanfermeijer et al., 1999). **Subpanel III**. To 1 mL of buffer was added 10 μL of 53 μM SBP and 1.5 μL of 100 mM CrO_4^{2-}. Subsequently, SO_4^{2-} was added from 50 mM stocks (▲). Also shown are the measurements in which 1.5 μL of 1 M SO_4^{2-} was added at the start of the experiment (♦); these data were used to correct for bleaching effects. The K_D for SO_4^{2-} was calculated using the method of Kragh-Hansen (1983).*

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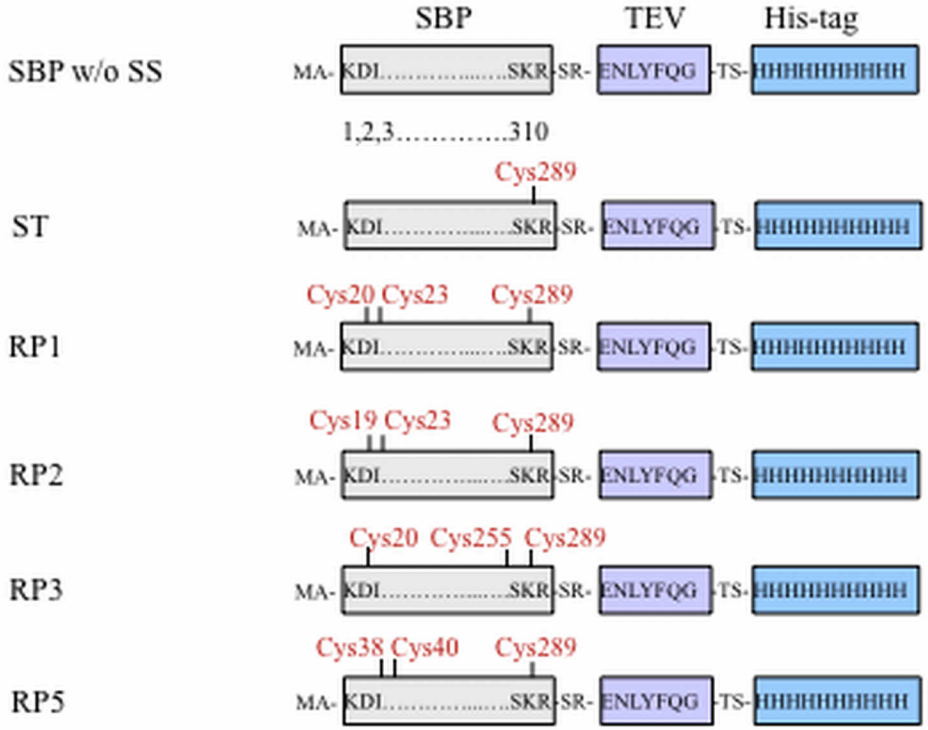
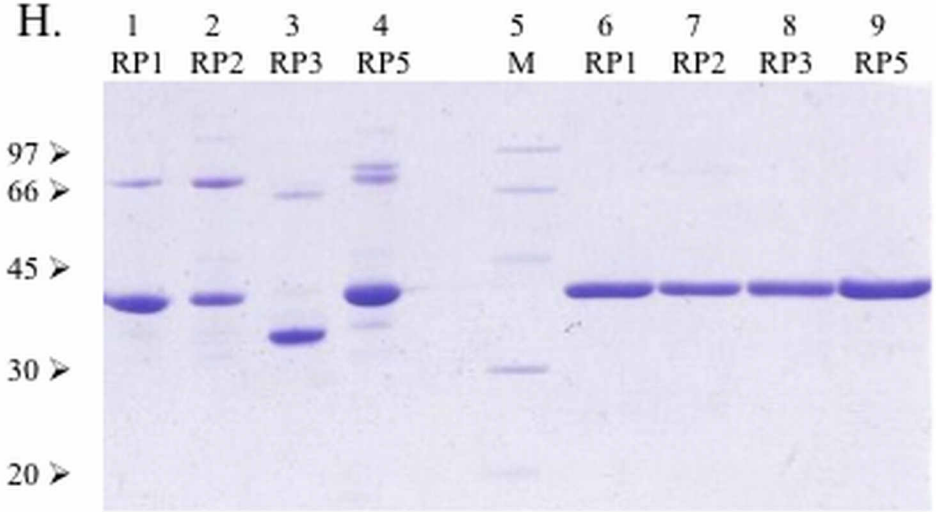
G.



***Panel G**: The amino acid sequence of SBP without SS and the positions of the cysteine mutations in the protein sequence.*

H.



***Panel H**: SDS-(12.5%) PAGE under non-reducing (Lane 1-4) and under reducing conditions (Lane 5-9). Mutant 3 migrates faster under non-reducing conditions due to intramolecular disulfide formation of Cys20 and Cys255.*
The proteins in lane 1-4 also show bands around 75 kDa, which reflect intermolecular dimer formation. The numbers at the left refer to the masses of the marker in kDa.

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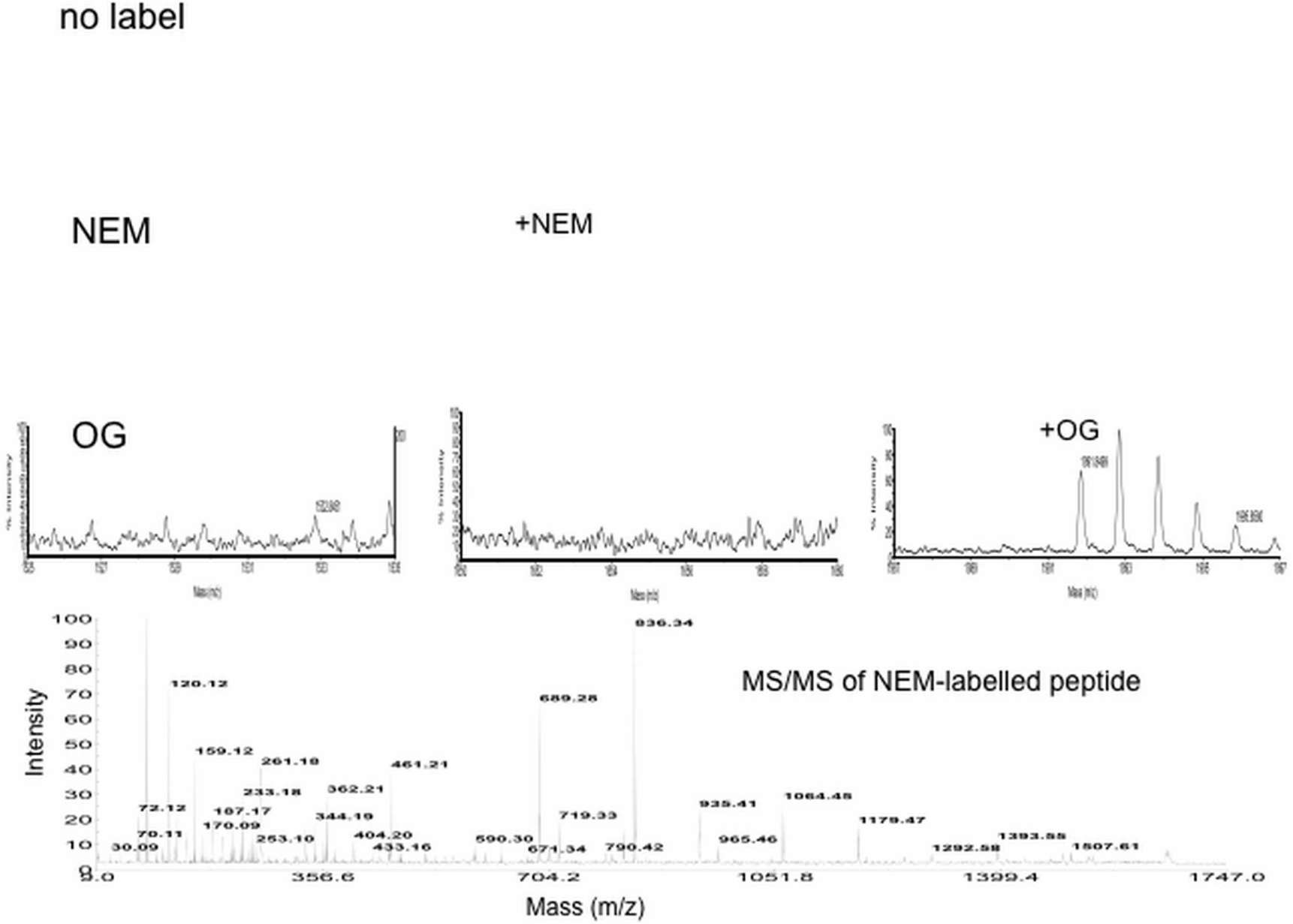
Target residues	Tryptic peptide sequence	Label	Mass difference	m/z
Cys 289	LFTIDEVF**C**GWAK	none	-	1528.75
Cys 289	LFTIDEVF**C**GWAK	NEM	+ 125.05	1653.80
Cys 289	LFTIDEVF**C**GWAK	OG	+ 463.05	1991.80
Cys 20, Cys 23	ELYE**C**YN**C**AFSAHWK	none	-	1861.76
Cys 20, Cys 23	ELYE**C**YN**C**AFSAHWK	2xNEM	+ 250.1	2111.86
Cys 20, Cys 23	ELYE**C**YN**C**AFSAHWK	2xOG	+ 926.1	2787.86

***Panel I**: Sequences and m/z values of cysteine-containing tryptic peptides from the RP1 mutant of the sulphate-binding protein (SB, functionalized wo or with NEM or Oregon Green (OG).*

Relevant mass spectra are shown in panel J.



***Panel J**: Mass spectra of peptide LFTIDEVFCGWAK, unlabelled (upper panels), NEM-labelled (middle panels) and OG-labelled (lower panels); MS/MS spectrum of the NEM-labelled peptide is shown in the bottom panel.*